

Engineering mitochondrial metabolic networks to increase yield and water use efficiency in *Camelina sativa*

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Project Goals:

Our research plan aims to establish the non-food oilseed crop plant, *Camelina sativa*, as a commercially viable, dedicated biofuel and bioproducts feedstock. We will focus on improving seed and oil yields by employing an integrated genetic and metabolic systems approach to increase the rates of photosynthetic CO₂ capture and conversion to triacylglycerols (TAGs). The major limitation in widespread adoption of *Camelina* as an industrial oilseed crop is its modest oil yield. Our research will address yield directly by employing a tissue-specific and whole-plant systems approach to identify the major regulatory mechanisms that limit 1) carbon fixation in photosynthetically active source tissues (leaves), 2) the transport of fixed carbon from source to sink tissues (seeds), and 3) the allocation of fixed carbon to TAG production. Our overall objective is to achieve up to a 300% increase per hectare in oil production, thereby meeting the yield and cost targets of a competitive biofuels and bioproducts crop while retaining its advantages for growth in marginal environments.

Abstract:

Environmental conditions and metabolic regulatory mechanisms exert major constraints on plant growth and the productivity by limiting the levels of carbon capture in photosynthetic source tissues (leaves) and the fixed carbon available for transport and allocation to sink tissues (e.g. seeds). A major constraint on carbon capture is the competition between the productive carboxylation reaction and non-productive oxygenation catalyzed by Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) in the Calvin-Benson cycle of photosynthesis. Our research addresses this constraint by investigating how metabolic networks interact to achieve high rates of carbon fixation while removing the toxic products of oxygenation via photorespiratory metabolism. These studies utilize the model oilseed crop, *Camelina sativa* engineered with a mitochondrial metabolite transporter, Organellar Carrier Protein 1 (OCP1). Plants expressing OCP1 (*Camelina*^{OCP}) assimilate carbon dioxide 20-30% times faster, and show increased growth rates and 38-50% higher seed yields. As such, *Camelina*^{OCP} plants provide an excellent system to investigate the metabolic networks of central carbon metabolism in source tissues and to identify the control points that limit carbon capture and constrain photoassimilate export for seed production in a crop plant.

On the basis of preliminary studies, OCP1 is hypothesized to enhance carbon dioxide assimilation as part of a metabolic release valve to reduce the levels of metabolites that inhibit

photosynthesis by 1) accelerating their recycling through the photorespiratory pathway, 2) aiding in the transfer of excess reducing equivalents from chloroplasts to mitochondria for dissipation via respiration, or 3) participating in the dissipation of excess reducing equivalents by augmenting the uncoupling capacity of mitochondria. We will present the details on the photosynthetic parameters impacted by OCP1 activity using advanced gas exchange and chlorophyll fluorescence measurements. These studies are complemented membrane transport studies and ^{13}C metabolic flux data and to define the metabolic network dynamics responsible for increased carbon capture in *Camelina*^{OCP}.

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